#17

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	U.S. Patent No. 4,199,569
Issued.:	April 22, 1980
To:	John C. Chabala and Michael H. Fisher
For:	SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231
Attn: Box Patent Extension

APPLICATION FOR SECOND INTERIM EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156(e)(2)

Sir:

Your Applicant, Merck & Co., Inc. a corporation organized and existing under the laws of the state of New Jersey, represents that it is the assignee of the entire interest in and to Letters Patent of the United States No. 4,199,569 granted to John C. Chabala, and Michael H. Fisher on the 22nd day of April 1980 for SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF. Your Applicant, acting through its duly authorized attorney, hereby submits this application for interim extension of patent term under 35 USC§156(e)(2).

Applicants filed a request for the extension of U.S. Patent No. 4,199, 569 on 8 January 1997 within the period of time for doing so following the approval of the New Drug Application for STROMECTOL®. The normal expiration date of the patent was 3 October 1997.

Applicants then received copies of communications from Ms. Karin Tyson of the USPTO to Mr. Ronald Wilson of the United States Food and Drug Administration (FDA) dated 17 January 1997 confirming the patent expiration dates and from Mr. Ronald Wilson to Mr. Steven G. Kunin of the USPTO dated 7 March 1997 confirming the regulatory review period for STROMECTOL®.

On 13 August 1997, Applicants applied for a 1 year interim extension of US patent 4,199,569 under 35 USC 156(e)(2) since it appeared at that time that the final decision on the application for patent term extension, including the six month comment period, would not be completed by the expiration date of the patent, 3 October 1997.

On 12 September 1997, the Patent and Trademark Office granted one year interim extension to 3 October 1998.

Since the granting of the interim extension, Applicants have received copies of correspondence from Thomas J. McGinnis of the Food and Drug Administration to the Commissioner of Patent and Trademarks dated 22 June 1998 in which the regulatory review period for STROMECTOL® was reviewed and confirmed.

It appears that the completion of the procedure for the granting of the patent term extension, including the six month comment period will not be completed by the interim extended date of 3 October 1998. Applicants thus hereby request an additional one year interim extension to 3 October 1999. Applicants note that the complete requested term of patent extension is 2.8 years, thus this second interim one year extension, for a total of two years interim extension, will not extend beyond the full requested term of patent extension.

At this time, Applicants would like to apply for a second year interim extension of U.S. Patent 4,199,569 under 35 USC 156(e)(2) since the completion of the review of the application for patent term extension, including the six month comment period, will extend beyond the current interim extension date of 3 October 1998.

Thank you for your consideration of this matter.

Respectfully submitted,

By 
David L. Rose
Reg. No. 26,332
Attorney for Applicants
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907
(908) 594-4777

Date: July 31, 1998

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

JUN 22 1998

Re: STROMECTOL®
Docket No. 97E-0061

The Honorable Bruce Lehman
Assistant Secretary of Commerce and
Commissioner of Patents and Trademarks
Box Pat. Ext.
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Commissioner Lehman:

This is in regard to the application for patent term extension for U.S. Patent No. 4,199,569, filed by Merck & Co., Inc., under 35 U.S.C. § 156 et seq. We have reviewed the dates contained in the application and have determined the regulatory review period for STROMECTOL®, the human drug product claimed by the patent.

The total length of the regulatory review period for STROMECTOL® is 2,291 days. Of this time, 2,055 days occurred during the testing phase and 236 days occurred during the approval phase. These periods of time were derived from the following dates:

1. The date an exemption under subsection 505(i) of the Federal Food, Drug, and Cosmetic Act involving this drug product became effective: August 17, 1990.

   The applicant claims July 17, 1990, as the date the Investigational New Drug application (IND) became effective. However, FDA records indicate that the IND effective date was August 17, 1990, which was thirty days after FDA receipt of the IND.

2. The date the application was initially submitted with respect to the human drug product under subsection 505(b) of the Federal Food, Drug, and Cosmetic Act: April 1, 1996.

   The applicant claims March 29, 1996, as the date the New Drug Application (NDA) for STROMECTOL® (NDA 50-742) was initially submitted. However, FDA records indicate that NDA 50-742 was submitted on April 1, 1996.

3. The date the application was approved: November 22, 1996.

   FDA has verified the applicant's claim that NDA 50-742 was approved on November 22, 1996.

This determination of the regulatory review period by FDA does not take into account the effective date of the patent, nor does it exclude one-half of the testing phase as required by 35 U.S.C. § 156(c)(2).

Please let me know if we can be of further assistance.

Sincerely yours,

Thomas J. McGinnis

Thomas J. McGinnis, R.Ph.
Deputy Associate Commissioner
for Health Affairs

cc: David L. Rose
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SEP 15 1997

David L. Rose
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907

Re: Patent Term Extension
Application for
U.S. Patent No. 4,199,569

Dear Mr. Rose:

Your letter filed August 18, 1997, noting the necessity of an interim extension, is acknowledged. A copy of your letter will be forwarded to Mr. Wilson so that the Food and Drug Administration's records will be complete.

An order granting an interim extension under 35 U.S.C. § 156(e)(2) is enclosed extending the term of U.S. Patent No. 4,199,569 for a period of one year.

Telephone inquiries regarding this communication should be directed to the undersigned at (703)306-3159.

Karin Tyson
Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

cc: Ronald L. Wilson, Director
Health Assessment Policy Staff
Office of Health Affairs (HFY-20)
Food and Drug Administration
5600 Fishers Lane, Room 15-22
Rockville, MD 20857

RE: STROMECTOL®
FDA Docket No. 97E-0061

kt

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE COMMISSIONER OF PATENTS AND TRADEMARKS

In re Merck & Co., Inc.	:	
Request for Patent Term Extension	:	ORDER GRANTING
U.S. Patent No. 4,199,569	:	INTERIM EXTENSION

Merck & Co., Inc., the owner of record in the Patent and Trademark Office (PTO) of U.S. Patent No. 4,199,569, filed an application for patent term extension under 35 U.S.C. § 156 on January 8, 1997. The original term of the patent is due to expire on October 3, 1997. The patent claims the active ingredient ivermectin in the human drug product "STROMECTOL®," which was approved by the Food and Drug Administration for commercial marketing or use on November 22, 1996. An extension of 1,026 days is requested.

The initial PTO review of the application to date indicates that the subject patent would be eligible for extension of the patent term under 35 U.S.C. § 156. A final determination of the length of the extension of the patent term and issuance of a patent term extension certificate cannot be made until a final determination of the length of the regulatory review period is made. Since the original term of the patent would expire before a certificate of patent term extension can be issued, an interim extension of the patent term is appropriate.

An interim extension under 35 U.S.C. § 156(e)(2) of the term of U.S. Patent No. 4,199,569 is granted for a period of one year from October 3, 1997, the original expiration date of the patent.

SEP 12 1997

Date

Bruce A. Lehman
Assistant Secretary of Commerce and
Commissioner of Patents and Trademarks

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	U.S. Patent No. 4,199,569
Issued.:	April 22, 1980
To:	John C. Chabala and Michael H. Fisher
For:	SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231
Attn: Box Patent Extension

APPLICATION FOR INTERIM EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156(e)(2)

Sir:

Your Applicant, Merck & Co., Inc. a corporation organized and existing under the laws of the state of New Jersey, represents that it is the assignee of the entire interest in and to Letters Patent of the United States No. 4,199,569 granted to John C. Chabala, and Michael H. Fisher on the 22nd day of April 1980 for SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF. Your Applicant, acting through its duly authorized attorney, hereby submits this application for interim extension of patent term under 35 USC§156(e)(2).

Applicants filed a request for the extension of U.S. Patent No. 4,199, 569 on 8 January 1997 within the period of time for doing so following the approval of the New Drug Application for STROMECTOL®. The normal expiration date of the patent is 3 October 1997.

Applicants have received copies of communications from Ms. Karin Tyson of the USPTO to Mr. Ronald Wilson of the United States Food and Drug Administration (FDA) dated 17 January 1997 confirming the patent expiration dates and from Mr.

Application for Interim Extension of Patent Term
U.S. Patent No. 4,199,569
Page No.:

Ronald Wilson to Mr. Steven G. Kunin of the USPTO dated 7 March 1997 confirming the regulatory review period for STROMECTOL®.

At this time, Applicants would like to apply for a one year interim extension of U.S. Patent 4,199,569 since there will be a six month comment period following the publication of the initial request for an extension of the patent term.

Thank you for your consideration of this matter.

Respectfully submitted,

By _____
David L. Rose
Reg. No. 26,332
Attorney for Applicants
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907
(908) 594-4777

Date: August 13, 1997

MAR 1 1 1997

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Ronald L. Wilson, Director
Health Assessment Policy Staff
Office of Health Affairs (HFY-20)
Food and Drug Administration
5600 Fishers Lane, Room 15-22
Rockville, MD 20857

Re: STROMECTOL®

FDA Docket No. 97E-0061

Dear Mr. Wilson:

Transmitted herewith is a copy of the application for patent term extension of U.S. Patent No. 4,199,569. The application was filed on January 8, 1997, under 35 U.S.C. § 156.

The patent claims a product that was subject to regulatory review under the Federal Food, Drug and Cosmetic Act. Subject to final review, the subject patent is considered to be eligible for patent term restoration. Thus, a determination by your office of the applicable regulatory review period is necessary. Accordingly, notice and a copy of the application are provided pursuant to 35 U.S.C. § 156(d)(2)(A).

Telephone inquiries regarding this matter should be directed to the undersigned at (703)306-3159.

Karin Tyson
Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

cc: David L. Rose
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907

kt

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

MAR - 7 1997

Re: STROMECTOL®
Docket No. 97E-0061

Stephen G. Kunin
Deputy Assistant Commissioner for
Patent Policy and Projects
Office of the Assistant Commissioner for Patents
U.S. Patent and Trademark Office
Crystal Park Building 2, Suite 919
Washington, D.C. 20231

Dear Mr. Kunin:

This is in regard to the application for patent term extension for U.S. Patent No. 4,199,569 filed by Merck & Co., Inc. under 35 U.S.C. § 156. The human drug product claimed by the patent is STROMECTOL® (ivermectin), which was assigned New Drug Application (NDA) No. 50-742.

A review of the Food and Drug Administration's official records indicates that this product was subject to a regulatory review period before its commercial marketing or use, as required under 35 U.S.C. § 156(a)(4). Our records also indicate that it represents the first permitted commercial marketing or use of the product, as defined under 35 U.S.C. § 156(f)(1), and interpreted by the courts in Glaxo Operations UK Ltd. v. Quigg, 706 F. Supp 1224 (E.D. Va. 1989), aff'd, 894 F. 2d 392 (Fed. Cir. 1990).

The NDA was approved on November 22, 1996, which makes the submission of the patent term extension application on January 8, 1997, timely within the meaning of 35 U.S.C. § 156(d)(1).

Should you conclude that the subject patent is eligible for patent term extension, please advise us accordingly. As required by 35 U.S.C. § 156(d)(2)(A) we will then determine the applicable regulatory review period, publish the determination in the Federal Register, and notify you of our determination.

Please let me know if we can be of further assistance.

Sincerely,

Ronald L. Wilson

Ronald L. Wilson, Director
Health Assessment Policy Staff
Office of Health Affairs

cc: David L. Rose
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

JAN 17 1997

Ronald L. Wilson, Director
Health Assessment Policy Staff
Office of Health Affairs (HFY-20)
Food and Drug Administration
5600 Fishers Lane, Room 15-22
Rockville, MD 20857

Dear Mr. Wilson:

The attached application for patent term extension of U.S. Patent No. 4,199,569, was filed on January 8, 1997, under 35 U.S.C. § 156. U.S. Patent No. 4,199,569 issued on April 22, 1980 from an application that claimed priority under 35 U.S.C. § 120 to an application that was filed on October 3, 1977. Accordingly, the original expiration date of the patent is October 3, 1997.

The assistance of your Office is requested in confirming that the product identified in the application, STROMECTOL® (ivermectin), has been subject to a regulatory review period within the meaning of 35 U.S.C. § 156(g) before its first commercial marketing or use and that the application for patent term extension was filed within the sixty-day period after the product was approved. Since a determination has not been made whether the patent in question claims a product which has been subject to the Federal Food, Drug and Cosmetic Act, this communication is NOT to be considered as notice which may be made in the future pursuant to 35 U.S.C. § 156(d)(2)(A).

Our review of the application to date indicates that the subject patent would be eligible for extension of the patent term under 35 U.S.C. § 156. However, processing of the application cannot be completed before the patent is due to expire, October 3, 1997. Accordingly, should you confirm that the product has been subject to a regulatory review period within the meaning of 35 U.S.C. § 156(g) before its first commercial marketing or use and that the application for patent term extension was timely filed, an interim extension under 35 U.S.C. § 156(e)(2) will be granted to provide sufficient time for processing of the application to be completed.

Telephone inquiries regarding this communication should be directed to the undersigned at (703) 306-3159.

Karin Tyson
Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Project

cc: David L. Rose
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re: U.S. Patent 4,199,569

Issued: April 22, 1980

To: John C. Chabala and Michael H. Fisher

For: SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS SND DERIVATIVES THEREOF

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

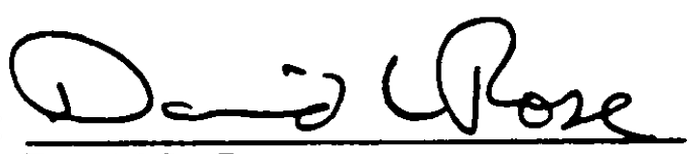
Re: Deposit Account 13-2755
MERCK & CO., Inc.
U.S. Patent 4,199,569

Sir:

Transmitted herewith is the application for extension of patent term under 35 U.S.C. 156 with regard to U.S. Patent 4,199,569.

Please charge our Deposit Account No. 13-2755 in the amount of $1,090.00. The Commissioner is hereby authorized to charge any additional fees, which may be required, or credit any overpayment to Account No. 13-2755. Duplicate copies of this sheet are enclosed.

Respectfully submitted,

By: 

David L. Rose
Reg. No. 26,332
Attorney for Applicant(s)

MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
(908) 594-4365

Date: 6 January, 1997

IN TRIPLICATE

(2)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re: U.S. Patent 4,199,569

Issued: April 22, 1980

To: John C. Chabala and Michael H. Fisher

For: SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS SND DERIVATIVES THEREOF

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

Re: Deposit Account 13-2755
MERCK & CO., Inc.
U.S. Patent 4,199,569

Sir:

Transmitted herewith is the application for extension of patent term under 35 U.S.C. 156 with regard to U.S. Patent 4,199,569.

Please charge our Deposit Account No. 13-2755 in the amount of $1,090.00. The Commissioner is hereby authorized to charge any additional fees, which may be required, or credit any overpayment to Account No. 13-2755. Duplicate copies of this sheet are enclosed.

Respectfully submitted,

By: David L. Rose

David L. Rose
Reg. No. 26,332
Attorney for Applicant(s)

MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
(908) 594-4365

Date: 6 January, 1997

IN TRIPLICATE

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re: U.S. Patent 4,199,569

Issued: April 22, 1980

To: John C. Chabala and Michael H. Fisher

For: SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS SND DERIVATIVES THEREOF

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

Re: Deposit Account 13-2755
MERCK & CO., Inc.
U.S. Patent 4,199,569

Sir:

Transmitted herewith is the application for extension of patent term under 35 U.S.C. 156 with regard to U.S. Patent 4,199,569.

Please charge our Deposit Account No. 13-2755 in the amount of $1,090.00. The Commissioner is hereby authorized to charge any additional fees, which may be required, or credit any overpayment to Account No. 13-2755. Duplicate copies of this sheet are enclosed.

Respectfully submitted,

By: David L. Rose

David L. Rose
Reg. No. 26,332
Attorney for Applicant(s)

MERCK & CO., INC.
P.O. Box 2000
Rahway, New Jersey 07065-0907
(908) 594-4365

Date: 6 January, 1997

IN TRIPLICATE

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	U.S. Patent No. 4,199,569
Issued.:	April 22, 1980
To:	John C. Chabala and Michael H. Fisher
For:	SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231
ATTN: Box Patent Extension

APPLICATION FOR EXTENSION OF PATENT
TERM UNDER 35 U.S.C. 156

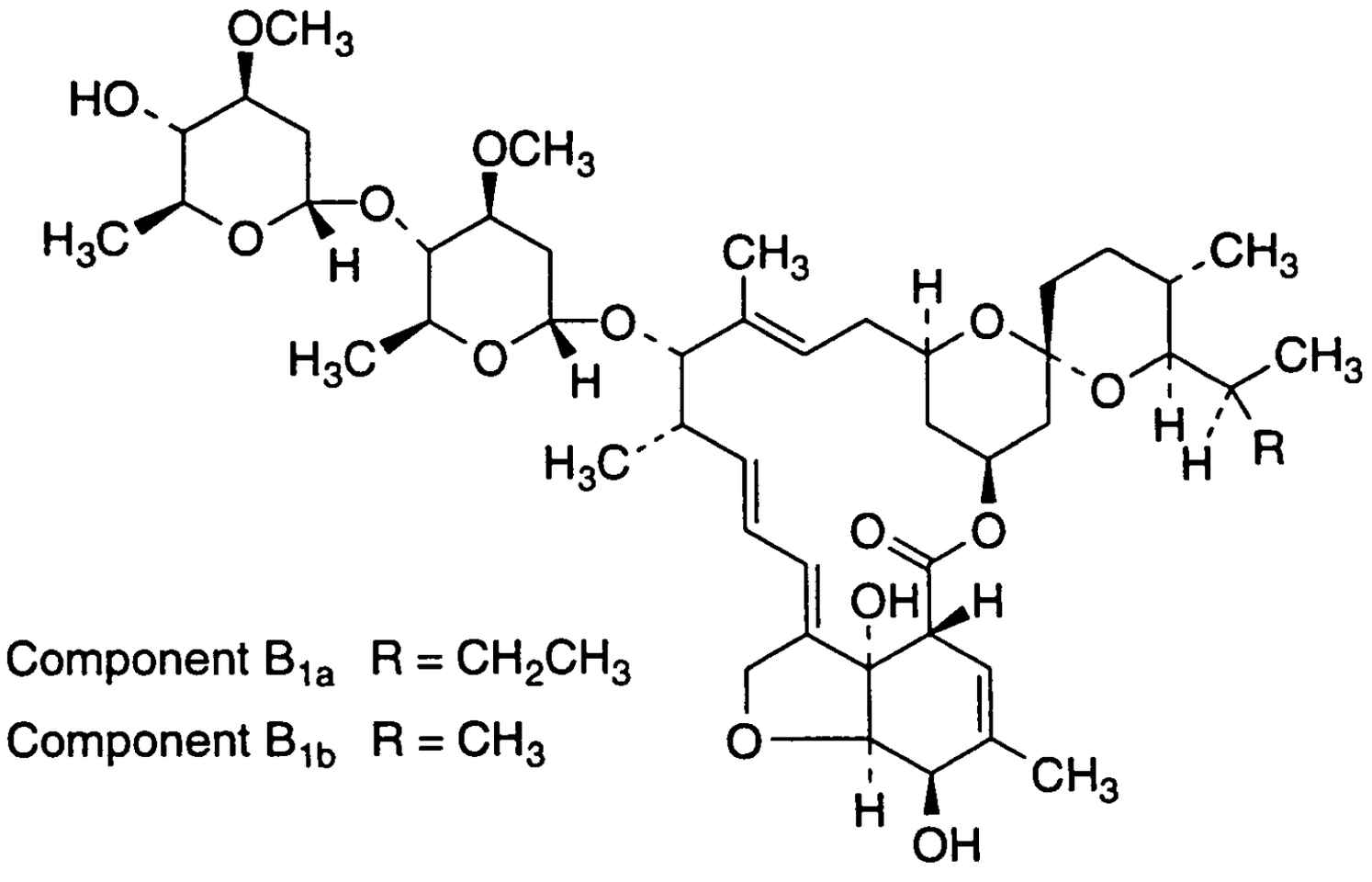
Sir:

Your Applicant, Merck & Co., Inc., a corporation organized and existing under the laws of the state of New Jersey, represents that it is the assignee of the entire interest in and to Letters Patent of the United States No. 4,199,569 granted to John C. Chabala and Michael H. Fisher on the 22nd day of April, 1980 for SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF by virtue of an assignment in favor of Merck & Co., Inc. recorded October 29, 1979, Reel 3697 Frame 070. Your Applicant, acting through its duly authorized attorney, hereby submits this application for extension of patent term under 35 U.S.C. 156 by providing the following information required by the rules promulgated by the U.S. Patent and Trademark Office (37 C.F.R. 1.740). For the convenience of the Patent and Trademark Office, the information contained in this application will be presented in a format which will follow the requirements of Section 1.740 of Title 37 of the Code of Federal Regulations,

(1) STROMECTOL® which contains as the active ingredient, ivermectin, consists of at least 90% by weight of the Bla component and no more than 10% of the Blb component. The chemical name for each component is as follows:

B1a = 22,23-dihydroavermectin Bla; or 5-O-demethyl-22,23-dihydro avermectin A1a.

B1b = 22,23-dihydroavermectin B1b; or 5-O-demethyl-22,23-dihydro avermectin A1b.



Component B_{1a} R = CH_2CH_3

Component B_{1b} R = CH_3

(2) The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act Section 507 (21 U.S.C. 357).

(3) The approved product, STROMECTOL®, received permission for commercial marketing or use under section 507 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 357) on 22 November, 1996.

(4) The only active ingredient in STROMECTOL® is ivermectin which has not been approved for commercial marketing or use under Section 507 of the

Federal Food, Drug and Cosmetic Act prior to the approval of NDA 50-742 (originally filed as NDA 20-721) by the Food and Drug Administration on 22 November, 1996.

(5) This application for extension of patent term under 35 U.S.C. 156 is being submitted within the permitted 60 day period pursuant to 37 C.F.R. 1.720(f), said period which will expire on 21 January, 1997.

(6) The complete identification of the patent for which extension is being sought is as follows:

Inventors:	John C. Chabala and Michael H. Fisher
Patent Number:	U.S. Patent 4,199,569
Issue Date:	April 22, 1980
Expiration Date:	October 3, 1997, as determined by 35 U.S.C. 154(c) enacted pursuant to the General Agreement of Tariffs and Trade (GATT), [Pub. L. No. 103-465 (H.R.5110), signed December 8, 1994, effective January 1, 1995]. Note, the original expiration date of the patent, prior to 35 U.S.C. 154(c) implementation, would be April 22, 1997.

(7) See "Attachment A" for a complete copy of the patent identified in paragraph (6) hereof.

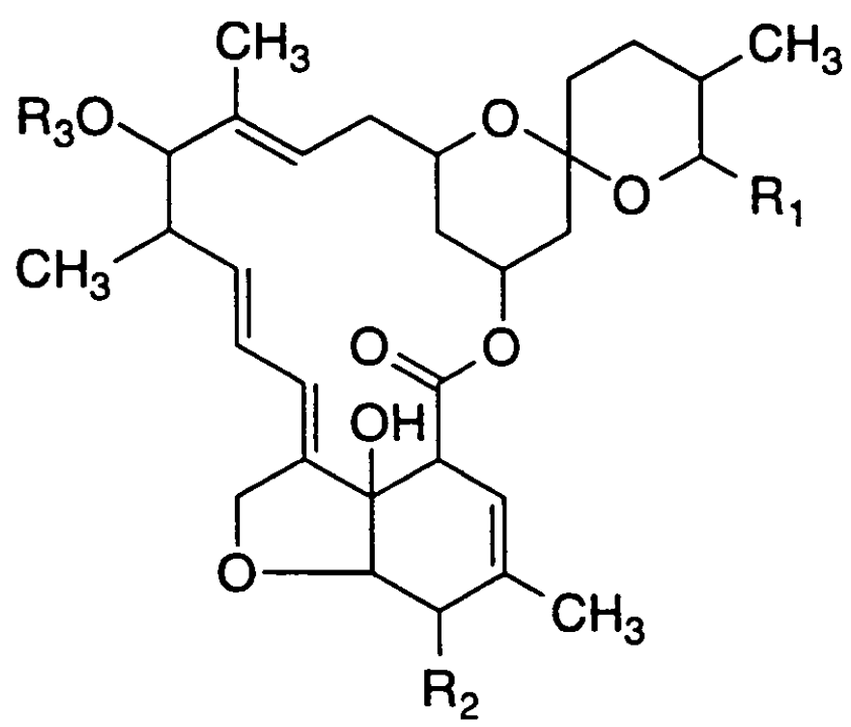
(8) No Terminal Disclaimer has been issued with regard to U.S. Patent 4,199,569. No Certificate of Correction or Re-examination Certificate has been issued with regard to U.S. Patent 4,199,569. Since U.S. Patent 4,199,569 isued on an application filed prior to December 12, 1980, no maintenance fees are payable for such patent.

(9) U.S. Patent 4,199,569 claims the approved product. Specifically, the active ingredient, ivermectin, is claimed in Claims 1, 2, 3, 4 and 6; the method of treatment utilizing the active ingredient, ivermectin, is claimed in Claims 15, 16 and 17; and the pharmaceutical composition containing the active ingredient, ivermectin, is claimed in Claims 18, 19 and 20.

Throughout the patent, the compounds disclosed therein are referred to as "C-076" compounds. The term "C-076" was used to generally identify the compounds prior to the adoption of the current terminology where the compounds are referred to as "avermectin" compounds.

Claim 1 reads as follows:

1. A compound having the formula:



wherein

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy, hydroxy or loweralkanoyloxy; and

R_3 is hydrogen; loweralkanoyl; α-L-oleandrosyl; 4'-loweralkanoyl-α-L-oleandrosyl; 4'(α-L-oleandrosyl)-α-L-oleandrosyl, 4"-loweralkanoyl-4'-(α-L-oleandrosyl)-α–L-oleandrosyl.

The approved product contains ivermectin which is a mixture of the B1a and B1b components which is a compound of Claim 1 wherein R_1 is sec-butyl for the B1a component and R_1 is isopropyl for the B1b component; R_2 is hydroxy; and R_3 is 4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

Claim 2 reads as follows:

2. The compound of Claim 1 wherein:

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy or hydroxy; and

R_3 is hydrogen, α-L-oleandrosyl or 4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

The approved product contains ivermectin which is a mixture of the B1a and B1b components which is a compound of Claim 2 wherein R_1 is sec-butyl for the B1a component and R_1 is iso-propyl for the B1b component; R_2 is hydroxy and R_3 is 4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

Claim 3 reads as follows:

3. The compound of Claim 2 wherein R_1 is iso-propyl.

The approved product contains ivermectin which is a mixture of the B1a and B1b components which is claimed in Claim 3 as the B1b component wherein R_1 is isopropyl.

Claim 4 reads as follows:

4. The compound of Claim 2 wherein R_2 is sec-butyl.

The approved product contains ivermectin which is a mixture of B1a and B1b components which is claimed in Claim 4 as the B1a component wherein R_1 is sec-butyl.

Claim 6 reads as follows:

6. The compound of Claim 4 which is 22,23-dihydro-C-076 B1a.

The approved product contains ivermectin which is a mixture of B1a and B1b components which is claimed in Claim 6 as the B1a component which is 22,23-dihydro C-076 B1a.

Claim 15 reads as follows:

15. A method for treating for parasites which comprises treating the animal or area infected with parasites with an effective amount of one or more compounds of Claim 1.

The approved product is used for the treatment of parasites by treating an animal infected with parasites with ivermectin which is a mixture of the B1a and B1b components which is a compound of Claim 1 wherein R_1 is sec-butyl for the B1a component and R_1 is iso-propyl for the B1b component; R_2 is hydroxy; and R_3 is 4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

Claims 16 reads as follows:

16. The method of Claim 15 wherein the active compound is C-076 B1a.

The approved product is used for the treatment of parasites by treating an animal infected with parasites with ivemectin which is a mixture of at least 90% of the B1a and no more than 10% of the B1b component which is claimed in Claim 16 as the B1a component. This definition covers the mixture defined in the approved product.

Claim 17 reads as follows:

17. The method of Claim 15 wherein the active compound is a mixture of about 80% C-076 B1a and 20% C-076 B1b.

The approved product is used for the treatment of parasites by treating an animal infected with parasites with ivermectin which is a mixture of the B1a and B1b components claimed in Claim 17 as a mixture of about 80% the C-076 B1a component and 20% of the C-076 B1b component.

Claim 18 reads as follows:

18. A composition for the treatment of parasitic infections which comprises an inert carrier and one or more compounds of Claim 1.

The approved product is an antiparasitic formulation used for the treatment of parasites, containing ivermectin which is a mixture of B1a and B1b components which is a compound of Claim 1 wherein R_1 is sec-butyl for the B1a component and R_1 is iso-propyl for the B1b component; R_2 is hydroxy; and R_3 is 4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

Claim 19 reads as follows:

19. The composition of Claim 18 wherein the active compound is C-076 B1a.

The approved product is an antiparasitic formulation used for the treatment of parasites, containing ivermectin which is a mixture of B1a and B1b components which is claimed in Claim 19 as the B1a component.

Claim 20 reads as follows:

20. The composition of Claim 18 wherein the active compound is a mixture of about 80% C-076 B1a and 20% C-076 B1b.

The approved product is an antiparasitic formulation used for the treatment of parasites, containing ivermectin which is a mixture of at least 90% of the B1a and no more than 10% of the B1b component which is claimed in Claim 20 as a mixture of about 80% of the C-076 B1a component and 20% of the C-076 B1b component. This definition covers the mixture defined in the approved product.

(10) The relevant dates and information pursuant to 35 U.S.C. 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period as follows:

(i) Investigational New Drug Application (IND 35,136) for ivermectin used for the treatment of strongyloidiasis and onchocerciasis was filed on 17 July 1990. An earlier Investigational New Drug Application (IND 24,923) had been filed on 13 September 1984 for ivermectin for the treatment of onchocerciasis only. No formal clinical trials were carried out under this IND and the IND was inactivated 28 December 1990. Patients were treated under IND 24,923 under a compassionate use protocol. None of the pendency period of IND 24,923 prior to the filing of IND 35,136 is being used in the determination of the duration of patent term restoration for STROMECTOL®. Only the regulatory review period beginning with the filing of IND 35,136 is being used for the determination of the period of patent term restoration for STROMECTOL® .

(ii) New Drug Application (NDA 20-721) for STROMECTOL® (ivermectin) was submitted on 29 March, 1996 (NDA 20-721 was changed to NDA 50-742 on 23 July 1996 to reflect a change in classification from Section 505 (21 USC 355) to Section 507 (21 USC 357)); and

(iii) New Drug Application (NDA 50-742) for STROMECTOL® (ivermectin) was approved on 22 November, 1996.

(11) As a brief description of the activities undertaken by Applicant, Merck & Co., Inc., during the applicable regulatory review period, attached hereto as "Attachment B" is a chronology of the major activities undertaken by Applicant in seeking the approval of STROMECTOL® and the major communications between the Applicant and the FDA from 17 July, 1990 to 22 November, 1996.

(12)(A) Applicant is of the opinion that U.S. Patent 4,199,569 is eligible for extension under 35 U.S.C. 156 because it satisfies all of the requirements for such extension as follows:

(a) 35 U.S.C. 156(a)
U.S. Patent 4,199,569 claims a product and a method of using a product.

(b) 35 U.S.C. 156(a)(1)
The term of the U.S. Patent 4,199,569 has not expired before submission of this application.

(c) 35 U.S.C. 156(a)(2)
The term of U.S. Patent 4,199,569 has never been extended under this provision of the law.

(d) 35 U.S.C. 156(a)(3)
The application for extension is submitted by the owner of record in accordance with the requirement of 35 U.S.C. 156(d) and rules of the U.S. Patent and Trademark Office.

(e) 35 U.S.C. 156(a)(4)
The product, STROMECTOL®, has been subjected to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. 156(a)(5)(A)
The commercial marketing or use of the product, STROMECTOL®, after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 357) under which such regulatory review period occurred.

(g) 35 U.S.C 156(c)(4)
No other patent has been extended for the same regulatory review period for the product, STROMECTOL®.

(B) The length of extension of the patent term of U.S. Patent 4,199,569 claimed by Applicant is 1026 days or 2.8 years. The length of the extension was determined pursuant to 37 C.F.R. 1.775 as follows:

(a) The regulatory review period under 35 U.S.C. 156(g)(B) began on 17 July, 1990 and ended on 22 November 1996 which is a total of 2321 days or 6.36 years which is the sum of (i) and (ii) below:

(i) The period of review under 35 U.S.C. 156(g)(2)(B)(i), the "Testing Period", began on 17 July 1990 and ended on 28 March 1996, which is 2082 days or 5.7 years and

(ii) The period of review under 35 U.S.C. 156(g)(2)(B)(ii), the "Application Period", began on 29 March, 1996 and ended on 22 November 1996 which is 239 days or 0.65 years;

(b) The regulatory review period upon which the period of extension is calculated is the entire regulatory review period as determined in sub-paragraph 12(B) above (2321 days) less:

(i) The number of days in the regulatory review period which were on or before the date on which the patent issued (April 22, 1980) which is 0 days, and

(ii) The number of days during the period from 13 December, 1993 to 3 May, 1995 during which the Applicant was reconsidering whether or not to continue to pursue the application for STROMECTOL® for stongylidiasis and onchocerciasis which is 507 days; and

(iii) One-half the difference between the number of days determined in sub-paragraph 12(B)(a)(i) (2082 days) and the number of days determined in sub-paragraph 12(B)(b)(ii) (507 days) which is 1575 days, one-half of which is 788 days;

(iv) The regulatory review period is calculated by subtracting the number of days determined in sub-paragraph 12(B)(b)(ii) and the number of days determined in sub-paragraph 12(B)(b)(iii) from the entire regulatory review period as determined in sub-paragraph 12(B) (which is 2321 days - 507 days - 788 days) which equals 1026 days.

(c) The number of days as determined in sub-paragraph 12(B)(b)(iv) (1026 days) when added to the original term of the patent (October 3, 1997, as determined by 35 U.S.C. 154(c)) would result in the date, 25 July 2000;

(d) Fourteen (14) years when added to the date of NDA approval (22 November 1996) would result in the date, 22 November 2010.

(e) The earlier date as determined in sub-paragraphs 12(B)(c) and 12(B)(d) is 25 July 2000;

(f) Since the original patent was issued before 24 September 1984, but a request for an exemption was not submitted before 24 September, 1984 and the commercial marketing or use of the product was not approved before 24 September, 1984, five (5) years when added to the original expiration date of the patent (October 3, 1997) would result in the date, October 3, 2002;

(g) The earlier date as determined in sub-paragraph (13)(e) and (13)(f) is 25 July 2000.

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) The prescribed fee for receiving and acting upon this application is to be charged to Deposit Account of Applicant is authorized in the attached letter, which is submitted in duplicate.

(15) Correspondence related to this application for extension of the patent term of U.S. Patent 4,199,569 should be addressed to David L. Rose. Reg. No. 26,332, Merck & Co., Inc. P.O. Box 2000, Rahway, New Jersey 07065-0907. Telephone (908) 594-4777.

(16) The instant application for extension of the patent term of U.S. Patent 4,199,569 is being submitted as one original and triplicate copies thereof.

(17) The requisite declaration pursuant to 37 C.F.R. 1.740(b) is attached.

Respectfully submitted,

By _____
David L. Rose
Reg. No. 26,332
Attorney for Applicants
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907
(908) 594-4365

Date: 6 January, 1997

CERTIFICATION

The undersigned hereby certifies that this application for extension of patent term under 35 U.S.C. 156 including its attachments and supporting papers is being submitted as one original and triplicate copies thereof.

_____
David L. Rose

Date: 6 January 1997

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	U.S. Patent 4,199,569
Issued:	April 22, 1980
To:	John C. Chabala and Michael H. Fisher
For:	SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

DECLARATION

Sir:

The undersigned Attorney for Merck & Co., Inc. which is the Applicant for Extension of Patent Term under 35 U.S.C. 156 with regard to U.S. Patent No. 4,199,569 hereby declares as follows:

(1) THAT he is a patent attorney authorized to practice before the Patent and Trademark Office and has general authority from the owner to act on behalf of the owner in patent matters;

(2) THAT he has reviewed and understands the contents of the application being submitted pursuant to 35 U.S.C. 156 and 37 C.F.R. 1.740;

(3) THAT he believes the patent is subject to extension pursuant to 35 U.S.C. 156 and 37 C.F.R. 1.710.

(4) THAT he believes an extension of the length claimed is fully justified under 35 U.S.C. 156.

(5) THAT he believes the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 35 U.S.C. 156 and 37 C.F.R. 1.720.

The undersigned hereby declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any extension of patent term issuing thereon.

Further declarant sayeth not.

Signed this 6 day of January, 1997.

David L. Rose

ATTACHMENT

A

United States Patent [19]

Chabala et al.

[11] **4,199,569**

[45] **Apr. 22, 1980**

[54] **SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF**

[75] Inventors: John C. Chabala, Westfield; Michael H. Fisher, Bridgewater, both of N.J.

[73] Assignee: Merck & Co., Inc., Rahway, N.J.

[21] Appl. No.: 928,111

[22] Filed: Jul. 31, 1978

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 838,603, Oct. 3, 1977, abandoned.

[51] Int. Cl.[2] A61K 31/70; C07H 17/08

[52] U.S. Cl. ..... 424/180; 536/9; 536/17 A; 260/343.41

[58] Field of Search 536/9, 17; 260/343.41

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,853,842	12/1974	Kishi et al.	536/9
3,950,360	4/1976	Aoki et al.	260/343.2 R

OTHER PUBLICATIONS

Chem. Abstract Cit. 86 (1977), 42838k.

Mishima, H. et al., Tetrahedron Letters, 10, pp. 711-714 (1975).

Journal of Antibiotics, 29(g), 1976, pp. 76-34, 76-42, 76-14, 76-16.

Primary Examiner—Johnnie R. Brown

Assistant Examiner—Blondel Hazel

Attorney, Agent, or Firm—David L. Rose; Harry E. Westlake

[57] **ABSTRACT**

Derivatives of C-076 are described in which the C-076 molecule, as series of macrolides, has a specific unsaturation, at the 22,23-position, catalytically reduced. Further reaction of the reduced C-076 compounds are also possible. The compounds thus produced have profound anthelmintic, insecticidal, ectoparasiticidal and acaracidal activity. Compositions containing the described C-076 derivatives as the active ingredient thereof are also disclosed.

20 Claims, No Drawings

SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

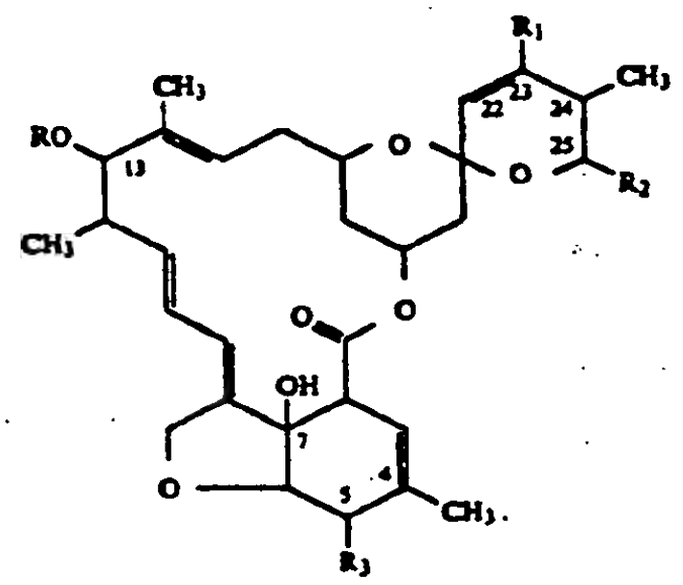
This application is a continuation-in-part of our copending application Ser. No. 838,603, filed Oct. 3, 1977 now abandoned.

BACKGROUND OF THE INVENTION

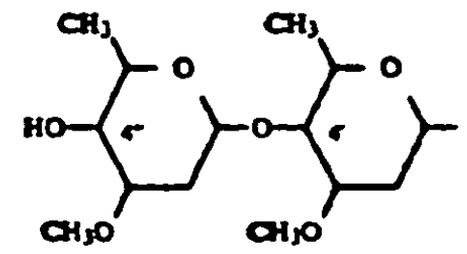
The term C-076 is used to describe a series of compounds isolated from the fermentation broth of a C-076 producting strain of *Streptomyces avermitilis*. The morphological characteristics of the culture are completely described in copending U.S. application Ser. No. 772,601. The C-076 compounds are a series of macrolides, each of which is substituted thereon at the 13-position with a 4-(α-L-oleandrosyl)-α-L-oleandrose group. The 1-series of C-076 compounds also has a 22,23-double bond, as well as several other double bonds. The selective reduction of the 22,23-double bond, without affecting the remaining double bonds is the subject matter of the instant application. The C-076 compounds and the instant derivatives thereof have a very high degree of anthelmintic and antiparasitic activity.

SUMMARY OF THE INVENTION

The C-076 series of compounds have the following structure:



wherein R is the 4'-(α-L-oleandrosyl)-α-L-oleandrose group of the structure:



and wherein the broken line indicates a single or a double bond;

R_1 is hydroxy and is present only when said broken line indicates a single bond;

R_2 is iso-propyl or sec-butyl; and

R_3 is methoxy or hydroxy.

There are eight different C-076 compounds and they are given the designations A1a, A1b, A2a, A2b, B1a, B1b, B2a, B2b based upon the structure of the individual compounds.

In the foregoing structural formula, the individual C-076 compounds are as set forth below.

	R_1	R_2	R_3
A1a	Double bond	sec-butyl	—OCH_3
A1b	Double bond	iso-propyl	—OCH_3
A2a	—OH	sec-butyl	—OCH_3
A2b	—OH	iso-propyl	—OCH_3
B1a	Double bond	sec-butyl	—OH
B1b	Double bond	iso-propyl	—OH
B2a	—OH	sec-butyl	—OH
B2b	—OH	iso-propyl	—OH

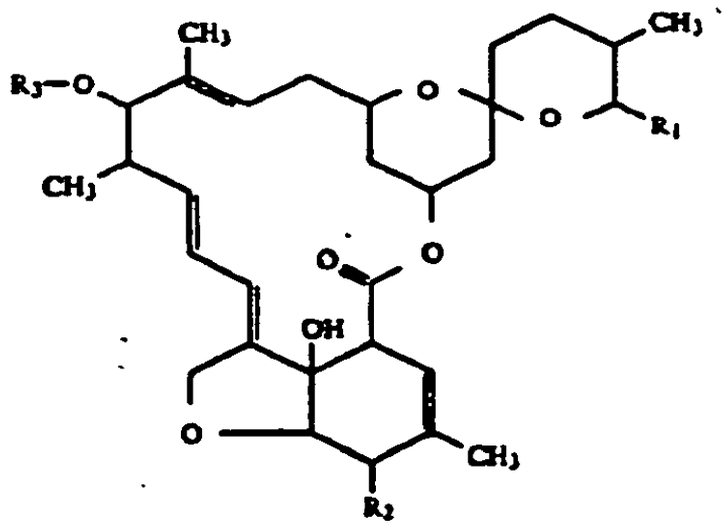
The C-076 compounds with the 22,23-unsaturations are identified as the "1-series" and it is only these compounds which are reduced to prepare the instant derivatives. Either before or after the reduction of the 22,23-double bond further reactions may be carried out in which one or both of the α-L-oleandrose moieties are removed, or in which one or more of the available hydroxy groups are acylated.

Based on taxonomic studies, the microorganisms capable of producing these C-076 compounds are of a new species of the genus Streptomyces, which has been named *Streptomyces avermitilis*. One such culture, isolated from soil is designated MA-4680 in the culture collection of Merck & Co., Inc., Rahway, N.J. A C-076 producing sample of this culture has been deposited in the permanent culture collection of the Fermentation Section of the Northern Utilization Research Branch, U.S. Department of Agriculture at Peoria, Ill., and has been assigned the accession number NRRL 8165. A sample of NRRL 8165 has also been deposited, without restriction as to availability, in the permanent culture collection of the American Type Culture Collection at 12301 Parklawn Drive, Rockville, Md. 20852, and has been assigned the accession number ATCC 31,267.

The above microorganism is illustrative of a strain of *Streptomyces avermitilis* which can be employed in the production of the C-076 compounds. However, such description also embraces mutants of the above described microorganism. For example, those C-076 producing mutants which are obtained by natural selection or those producted by mutating agents including X-ray irradiation, ultraviolet irradiation, nitrogen mustard or like treatments are also included within the ambit of this invention.

One example of such an organism is a strain of *Streptomyces avermitilis* MA 4848 which was isolated after irradiation with ultraviolet light of *Streptomyces avermitilis* MA 4680. A lyophilized tube and a frozen vial of this culture has been deposited in the permanent culture collection of the American Type Culture Collection, and they have been assigned the accession numbers 31272 and 31271 respectively. Slightly higher fermentation yields of C-076 have been obtained using this frozen stock as inoculum.

Thus, the compounds of the instant invention have the following structural formula:



wherein

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy, hydroxy or loweralkanoyloxy;

R_3 is hydrogen; loweralkanoyl; α-L-oleandrosyl; 4'-loweralkanoyl-α-L-oleandrosyl; 4'-(α-L-oleandrosyl)-α-L-oleandrosyl; 4''-loweralkanoyl-4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

In the instant invention, the term "loweralkanoyl" is intended to include those alkanoyl groups of from 2 to 6 carbon atoms such as acetyl, propionyl, butyryl, pivaloyl and the like.

Preferred compounds of the instant invention are realized in the above structural formula when:

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy or hydroxy; and

R_3 is hydrogen α-L-oleandrosyl or 4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

Additional preferred compounds are realized when the "loweralkanoyl" group of R_3 is acetyl in the disaccharide, monosaccharide and aglycone compounds.

As is readily apparent from an analysis of the structure of the C-076 starting materials, there are five unsaturations in the 1-series of compounds. An object of the instant invention is to reduce the 22,23-double bond while not affecting the remaining four unsaturations or any other functional group present on the molecule. It is necessary to select a specific catalyst for the hydrogenation, one that will selectively hydrogenate the least hindered from among a series of unsaturations. The preferred catalyst for such a selective hydrogenation procedure is one having the formula:

$$[(R_4)_3P]_3RhX$$

wherein R_4 is loweralkyl, phenyl, or loweralkyl substituted phenyl and X is a halogen.

In the preferred catalyst R_4 is phenyl and X is chlorine, that is the compound tris(triphenylphosphine)-rhodium (I) chloride, which is also known as Wilkinson's homogeneous catalyst.

The reaction is carried out using a catalytic amount of the catalyst. The amount of catalyst is not critical and from 0.05 to 0.5 moles of the catalyst for each mole of starting material have been successfully employed. Molar ratios in the range of 0.25 to 0.40 are preferred.

The hydrogenation is carried out in a hydrogen atmosphere which may be either at atmospheric pressure or up to about 4 atmospheres pressure in a standard laboratory hydrogenation apparatus. A solvent is normally employed to dissolve both the starting materials and the catalyst. Preferred solvents are hydrocarbon solvents such as benzene, toluene, petroleum ether and other alkane hydrocarbons. The reaction is complete when the calculated amount of hydrogen has been taken up by the reaction. This will generally require from about 1 to 48 hours. The reaction may be carried out at from room temperature of about 75° C., however, room temperature is preferred. The hydrogenation products are isolated and purified by techniques known to those skilled in the art.

Other reactions may be carried out on the C-076 starting materials or on the hydrogenated products to prepare the compounds of this invention. While it is possible to complete all of the other reactions on the C-076 starting material and have the hydrogenation step as the final reaction, it is preferred to carry out the hydrogenation step first. Because the 22,23-double bond is somewhat susceptible to nucleophilic addition, reaction conditions for removing the sugar groups or acylating the hydroxy groups must be carefully controlled if the 22,23-double bond is present. If the 22,23-double bond is hydrogenated first, the subsequent sugar removal and acylation is rendered more facile.

Thus, the additional reactions which may be carried out to prepare the compounds of this invention are the selective removal of one or both of the α-L-oleandrosyl moieties or the selective acylation of the susceptible hydroxy groups.

The reaction conditions which are generally applicable to the preparation of both the monosaccharide and aglycone involve dissolving the C-076 compound or the hydrogenated C-076 compound in an aqueous acidic non-nucleophilic organic solvent, miscible with water, preferably dioxane, tetrohydrofuran, dimethoxyethane, dimethyl formamide, bis-2-methoxyethyl ether, and the like, in which the water concentration is from 0.1 to 20% by volume. Concentrated acid is added to the aqueous organic solvent to the extent of 0.01 to 10% by volume. The reaction mixture is generally stirred at about 20°-40° C., preferably at room temperature, for from 6 to 24 hours. The lower concentrations of acid, from about 0.01 to 0.1% will predominately produce the monosaccharide under the above reaction conditions. Higher acid concentrations, from about 1 to 10% will predominantly produce the aglycone under the above reaction conditions. Intermediate acid concentrations will generally produce mixtures of monosaccharide and aglycone. The products are isolated, and mixtures are separated by techniques such as column, thin layer preparative and high pressure liquid chromatography, and other known techniques.

The acids which may be employed in the above process include mineral acids and organic acids such as sulfuric, hydrohalic, phosphoric, trifluoroacetic, trifluoro methane sulfonic and the like. The hydrohalic acids are preferably hydrochloric or hydrobromic. The preferred acid in the above process is sulfuric acid.

A further procedure for the preparation of the monosaccharide or aglycone of the C-076 compounds or of the hydrogenated C-076 compounds utilizes a different solvent system for the monosaccharide and the aglycone. The procedure for the preparation of the monosaccharide uses 1% acid by volume in isopropanol at from 20°-40° C., preferably room temperature, for from 6 to 24 hours. For the preparation of the aglycone, 1% acid, by volume, in methanol under the foregoing reaction conditions has been found to be appropriate.

When this procedure is empioyed on the starting material (the compounds with the 22,23-double bond)

there is a possibility of nucleophilic addition to the double bond. If such occurs, chromatographic purification will remove the by-product in order to allow for further reactions.

The acids listed above are appropriate for this process, and again sulfuric acid is the preferred acid.

The above described compounds are isolated from the reaction mixture and mixtures of compounds are separated using techniques known to those skilled in this art, and in particular the chromatographic techniques described above.

The acylated compounds are prepared using acylation techniques in which the reaction conditions will vary, depending upon the reactivity of the hydroxy group being acylated. Where there is more than one hydroxy group to be acylated, different reaction conditions are employed to minimize the formation of mixtures.

The acrylation reagents employed are generally the halide, preferably the chloride, of the above loweralkanoyl groups. That is the loweralkanoyl halide reagent is generally employed.

In addition, the acylation reagent could be in the form of the anhydride or of the halo formate. In the case of reactions carried out with the halide reagents, it is often advantageous to include in the reaction mixture a basic compound capable of reacting with and neutralizing the hydrogen halide which is liberated during the course of the reaction. Tertiary amines are preferred such as triethylamine, pyridine, dimethylamino pyridine, diisopropyl ethylamine and the like. The basic compound is required in equimolar amounts relative to the numbered moles of hydrogen halide being liberated, however excess amounts, even using the basic compound as a solvent, are not detrimental.

In the case of the A1 compounds of C-076, or of the hydrogenated C-076 A1 compounds there is only a single hydroxy group, 4" hydroxy, which may be acylated. The formation of the monosaccharide or the aglycone still leaves only a single hydroxy group which may be acylated, that is the 4' or 13 hydroxy group.

In the case of the 4", 4' and 13 hydroxy groups of C-076 A1 compounds, the acylating reagent is dissolved in a suitable solvent, pyridine is preferred, and the acylating reagent added. The reaction is maintained at from 0° C. to room temperature for from 4 to 24 hours. The product is isolated using known techniques.

The B1 compounds have 2 available hydroxy groups: at the 4"(4' or 13) and the 5-positions. However, the two hydroxy groups have similar reactivities. When the reaction of the acylating agent in pyridine is carried out at about room temperature for from 4 to 24 hours, the diacyl compound is recovered. When the reaction is carried out at 0° C. a mixture of the 4"(4' or 13) and 5 monoacyl compounds are recovered. To recover individual compounds, the mixture is placed on a chromatographic column or a preparative layer chromatographic plate of alumina or silica gel and the individual compounds are readily isolated. In addition, techniques such as high pressure liquid chromatography may be employed to separate mixtures of acylated compounds.

The acyl compounds thus prepared are isolated from the reaction mixture using techniques known to those skilled in this art.

The novel compounds of this invention have significant parasiticidal activity as anthelmintics, ectoparasiticides, insecticides and acaricides, in human and animal health and in agriculture.

The disease or group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes widespread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagostomum, Chabertia, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterakis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris and Parascaris. Certain of these, such as Nematodirus, Cooperia, and Oesphagostomum attack primarily the intestinal tract while others, such as Haemonchus and Ostertagia, are more prevalent in the stomach while still others such as Dictyocaulus are found in the lungs. Still other parasites may be located in other tissues and organs of the body such as the heart and blood vessels, subcutaneous and lymphatic tissue and the like. The parasitic infections known as helminthiases lead to anemia, malnutrition, weakness, weight loss, severe damage to the walls of the intestinal tract and other tissues and organs and, if left untreated, may result in death of the infected host. The hydrogenated C-076 compounds of this invention have unexpectedly high activity against these parasites, and in addition are also active against Dirofilaria in dogs, Nematospiroides, Syphacia, Aspiculuris in rodents, arthropod ectoparasites of animals and birds such as ticks, mites, lice, fleas, blowfly, in sheep Lucilia sp., biting insects and such migrating diperous larvae as Hypoderma sp. cattle, Gastrophilus in horses, and Cuterebra sp. in rodents.

The instant compounds are also useful against parasites which infect humans. The most common genera of parasites of the gastro-intestinal tract of man are Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Trichuris, and Enterobius. Other medically important genera of parasites which are found in the blood or other tissues and organs outside the gastrointestinal tract are the filiarial worms such as Wuchereria, Brugia, Onchocerca and Loa, Dracunculus and extra intestinal stages of the intestinal worms Strongyloides and Trichinella. The compounds are also of value against arthropods parasitizing man, biting insects and other dipterous pests causing annoyance to man.

The compounds are also active against household pests such as the cockroach, Blatella sp., clothes moth, Tineola sp., carpet beetle, Attagenus sp., and the housefly *Musca domestica*.

The compounds are also useful against insect pests of stored grains such as Tribolium sp., Tenebrio sp. and of agricultural plants such as spider mites, (Tetranychus sp.), aphids, (Acyrthiosiphon sp.); against migratory orthopterans such as locusts and immature stages of insects living on plant tissue. The compounds are useful as a nematocide for the control of soil nematodes and plant parasites such as Meloidogyne spp. which may be of importance in agriculture.

These compounds may be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench where used as an anthelmintic in mammals. The drench is normally a solution, suspension or dispersion of the active ingredient usually in water together with a suspending agent such as bentonite and a

wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contains from about 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight. The capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or di-calcium phosphate.

Where it is desired to administer the C-076 derivatives in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like. Such unit dosage formulations may be varied widely with respect to their total weight and content of the antiparasitic agent depending upon factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or optionally fed separately. Alternatively, the antiparasitic compounds of our invention may be administered to animals parenterally, for example, by intraruminal, intramuscular, intratracheal, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil, cotton seed oil and the like. Other parenteral vehicles such as organic preparation using solketal, glycerol formal, and aqueous parenteral formulations are also used. The active monosaccharide or aglycone C-076 compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 5% by weight of the active compound.

Although the antiparasitic agents of this invention find their primary use in the treatment and/or prevention of helminthiasis, they are also useful in the prevention and treatment of diseases caused by other parasites, for example, arthropod parasites such as ticks, lice, fleas, mites and other biting insects in domesticated animals and poultry. They are also effective in treatment of parasitic diseases that occur in other animals including humans. The optimum amount to be employed for best results will, of course, depend upon the particular compound employed, the species of animal to be treated and the type and severity of parasitic infection or infestation. Generally good results are obtained with our novel compounds by the oral administration of from about 0.001 to 10 mg. per kg. of animal body weight, such total dose being given at one time or in divided doses over a relatively short period of time such as 1-5 days. With the preferred compounds of the invention, excellent control of such parasites is obtained in animals by administering from about 0.025 to 0.5 mg. per kg. of body weight in a single dose. Repeat treatments are given as required to combat re-infections and are dependent upon the species of parasite and the husbandry techniques being employed. The techniques for administering these materials to animals are known to those skilled in the veterinary field.

When the compounds described herein are administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant one that will not react with the antiparasitic agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, crushed limestone and the like. The active hydrogenated C-076 compounds are intimately dispersed throughout the carrier by methods such as grinding, stirring, milling or tumbling. Compositions containing from about 0.005 to 2.0% weight of the active compound are particularly suitable as feed premixes. Feed supplements, which are fed directly to the animal, contain from about 0.0002 to 0.3% by weight of the active compounds.

Such supplements are added to the animal feed in an amount to give the finished feed the concentration of active compound desired for the treatment and control of parasitic diseases. Although the desired concentration of active compound will vary depending upon the factors previously mentioned as well as upon the particular C-076 derivative employed, the compounds of this invention are usually fed at concentrations of between 0.00001 to 0.002% in the feed in order to achieve the desired antiparasitic result.

In using the compounds of this invention, the individual hydrogenated C-076 components may be prepared and used in that form. Alternatively, mixtures of two or more of the individual hydrogenated C-076 components may be used, as well as mixtures of the parent C-076 compounds other C-076 compound or other active compounds not related to C-076 and the compounds of this invention.

In the isolation of the C-076 compounds, which serve as starting materials for the instant processes, from the fermentation broth, the various C-076 compounds will be found to have been prepared in unequal amounts. In particular an "a" series compound will be prepared in a higher proportion than the corresponding "b" series compound. The weight ratio of "a" series to the corresponding "b" series is about 75:25 to 99:1. The differences between the "a" series and "b" series is constant throughout the C-076 compounds and consists of a sec-butyl group and an iso-propyl group respectively at the 25 position. This difference, of course, does not interfere with any of the instant reactions. In particular may not be necessary to separate the "b" components from the related "a" component. Separation of these closely related compounds is generally not practiced since the "b" compound is present only in a very small percent by weight, and the structural difference has negligible effect on the reaction processes and biological activities.

In particular it has been found that the starting materials for the compounds of this invention are very often

prepared in a ratio of about 80% C-076 B1a or A1a and 20% C-076 B1b or A1b. Thus the preferred composition of this invention is one which contains about 80% of the "a" component and 20% of the "b" component.

The C-076 compounds of this invention are also useful in combatting agricultural pests that inflict damage upon crops while they are growing or while in storage. The compounds are applied using known techniques as sprays, dusts, emulsions and the like, to the growing or stored crops to effect protection from such agricultural pests.

The following examples are provided in order that this invention might be more fully understood; they are not to be construed as limitative of the invention.

The hydrogenated C-076 derivatives prepared in the following examples are generally isolated as amorphous solids and not as crystalline solids. They are thus characterized analytically using techniques such as mass spectrometry, nuclear magnetic resonance, and the like. Being amorphous, the compounds are not characterized by sharp melting points, however, the chromatographic and analytical methods employed indicate that the compounds are pure.

EXAMPLE 1

22,23-Dihydro C-076 A1a

51.0 Mg of C-076 A1a and 14.4 mg. of tris (triphenylphosphine) rhodium (I) chloride are combined in 3.5 ml. of benzene and hydrogenated for 20 hours at room temperature under atmospheric pressure. The crude reaction mixture is chromatographed on a preparative layer chromatography plate eluting twice with 10% tetrahydrofuran in chloroform. The product is removed from the support using ethyl acetate which is evaporated to dryness and the residue analyzed with 300 MHz nuclear magnetic resonance and mass spectroscopy indicating the preparation of 22,23-dihydro C-076 A1a.

EXAMPLE 2

22,23-Dihydro C-076 B1a

The solution of 87.3 mg. of C-076 B1a in 6 ml. of benzene containing 25 mg. of tris (triphenylphosphine rhodium (I) chloride is hydrogenated for 4 hours at room temperature under 1 atmosphere of hydrogen pressure. Preparative layer chromatography on silica gel eluting with 20% tetrahydrofuran in chloroform recovers starting material. The sample is rehydrogenated following the above conditions for 19 hours. Preparative layer chromatography recovers 55 mg. of 22,23-dihydro C-076 B1a which is identified by mass spectrometry and 300 MHz nuclear magnetic resonance.

EXAMPLE 3

22,23-Dihydro C-076 B1a

A solution of 1.007 g. of C-076 B1a, 314 mg. of tris (triphenylphosphine) rhodium (I) chloride and 33 ml. of benzene is hydrogenated for 21 hours at room temperature under 1 atmosphere of hydrogen pressure. The solvent is removed in vacuo and the residue dissolved in a 1:1 mixture of methylene chloride and ethyl acetate and filtered. The filtrate is placed on a column of 60 g. of silica gel eluting with a 1:1 mixture of methylene chlorid and ethyl acetate taking 10 ml. fractions. Fractions 14–65 are combined and evaporated to dryness affording 1.118 g. of a solid material which is indicated by high pressure liquid chromatography to be a 60/40 mixture of the hydrogenated product and starting material. The mixture is rehydrogenated in 55 ml. of benzene adding 310 mg. of tris (triphenylphosphine) rhodium (I) chloride and stirring for 21 hours at room temperature under 1 atmosphere of hydrogen pressure. The solvent is removed in vacuo and the residue chromatographed on 80 g. of silica gel using 40:60 mixture of ethyl acetate and methylene chloride as eluant. 10 Ml. fractions are taken and the product appears in fractions 26–80. These fractions are combined and evaporated to dryness in vacuo affording a yellow oil. The oil is dissolved in benzene and lyophilized affording a pale yellow powder which is identified as 22,23-dihydro C-076 B1a by mass spectrometry and 300 MHz nuclear magnetic resonance. 0.976 G. of product is obtained.

EXAMPLE 4

22,23-Dihydro C-076 A1a Monosaccharide

11.2 Mg. of 22,23-dihydro C-076 A1a is dissolved in 1.1 ml. of 1% sulfuric acid in isopropanol and stirred for 20 hours at room temperature. The reaction mixture is diluted with chloroform to a volume of about 5.0 ml. and washed with saturated aqueous sodium bicarbonate solution and sodium chloride solution. The organic layer is dried over sodium sulfate and evaporated to dryness in vacuo affording an oil. The oil is placed on a silica gel preparative layer chromatography plate and eluted with 5% tetrahydrofuran in chloroform. The product is removed from the plate and lyophilized from benzene affording 5.2 mg. of a white powder which is identified by 300 MHz nuclear magnetic resonance and mass spectrometry as 22,23-dihydro C-076 A1a monosaccharide.

EXAMPLE 5

22,23-Dihydro C-076 A1a Aglycone

10.1 Mg. of 22,23-dihydro C-076 A1a is stirred for 20 hours in 1.1 ml. of 1% sulfuric acid in methanol at room temperature. The reaction mixture is treated as in Example 4 affording an oil which is purified by preparative layer chromatography on silica gel eluting with 5% tetrahydrofuran in chloroform. The product is removed from the chromatography plate and lyophilized from benzene affording 4.2 mg. of a white powder which 300 MHz nuclear magnetic resonance and mass spectrometry indicate to be 22,23-dihydro C-076 A1a aglycone.

EXAMPLE 6

22,23-Dihydro C-076 B1a Monosaccharide

395 Mg. of 22,23-dihydro C-076 B1a is added to a stirred solution of 50 ml. of 1% sulfuric acid in isopropanol and the solution is stirred for 14 hours at room temperature. The reaction mixture is treated as in Example 4 affording 0.404 g. of a foam after lyophilization from benzene. The foam is chromatographed on 6 preparative layer silica gel chromatography plates eluting twice with 4% tetrahydrofuran in chloroform. The monosaccharide with a Rf 0.15 is collected and washed from the silica gel with a total of 650 ml. of ethyl acetate. The combined washings are evaporated to dryness and the residue lyophilized from benzene to afford 0.2038 g. of 22,23-dihydro C-076 B1a monosaccharide which high pressure liquid chromatography indicates to be essentially pure.

EXAMPLE 7

22,23-Dihydro C-076 B1a Aglycone

9.7 Mg. of 22,23-dihydro C-076 B1a is stirred overnight in 1 ml. of a 1% sulfuric acid in methanol solution. The reaction mixture is treated as in Example 4 and the solid material treated with preparative layer chromatography on silica gel eluting with 10% tetrahydrofuran in chloroform. The oil recovered from the chromatography plate is lyophilized from benzene affording 4.7 mg. of a white powder which 300 MHz nuclear magnetic resonance and mass spectrometry indicate to be 22,23-dihydro C-076 B1a aglycone.

EXAMPLE 8

22,23-Dihydro C-076 B1a Aglycone

0.486 G. of 22,23-dihydro C-076 B1a is added to a stirred solution of 50 ml. of 1% sulfuric acid in methanol and the reaction mixture stirred for 13 hours at room temperature. The reaction mixture is diluted with 250 ml. of methylene chloride and washed with 50 ml. of saturated aqueous potassium bicarbonate and 50 ml. of water. The aqueous layer is washed twice with 20 ml. portions of methylene chloride and the combined organic phases are dried with saturated brine and sodium sulfate and evaporated to dryness in vacuo affording 0.480 g. of a pale yellow foam. The foam is dissolved in 4 ml. of methylene chloride and placed on 4 preparative layer chromatography silica gel plates and eluted 4 times with 4% tetrahydrofuran and chloroform. The product is recovered from the silica gel plates affording an oily residue which is lyophilized from benzene affording 255.8 mg. of a white solid. Traces of methyl oleandroside are indicated to be present in the solid material. The white solid is then lyophilized again from benzene and placed under high vacuum for 20 hours to remove the impurity affording 22,23-dihydro C-076 B1a aglycone.

EXAMPLE 9

4"-O-acetyl-22,23-Dihydro C-076 A1a

6.8 Mg. of 22,23-dihydro C-076 A1a is dissolved in 40 drops of anhydrous pyridine, chilled to 0° C. and treated with 20 drops of acetic anhydride. The reaction mixture is allowed to warm to room temperature and stirred overnight. The reaction mixture is diluted with 5 ml. of ether and 6 ml. of water and the layers separated. The aqueous phase is washed twice with ether and the organic layers combined and back washed 3 times with water. The ether layer is dried over magnesium sulfate and evaporated to dryness in vacuo affording an oil. The oil is chromatographed on silica gel preparative layer chromatography plates eluting with 5% tetrahydrofuran in chloroform. The product is recovered from the plates and lyophilized from benzene affording 6.1 mg. of 4"-O-acetyl-22,23-dihydro C-076 A1a as determined by mass spectrometry at 300 MHz nuclear magnetic resonance.

EXAMPLE 10

4"-O-acetyl-22,23-Dihydro C-076 B1a and 4",5-di-O-acetyl 22,23-Dihydro C-076 B1a

18.6 Mg. of 22,23-dihydro C-076 B1a is dissolved in 63 drops (about 1 ml.) of dry pyridine and treated with 9 drops of acetic anhydride at 0° C. The reaction is stirred under nitrogen for 6 hours at 0° C. The mixture is then quenched with 5 ml. of water and extracted 3 times with 3 ml. portions of ether. The combined ether extracts are then washed 3 times with 3 ml. portions of water and dried over magnesium sulfate and evaporated to dryness in vacuo. The oil is chromatographed on preparative layer silica gel chromatography plates eluting twice with 5% tetrahydrofuran in chloroform affording 5.8 mg. of 4"-O-acetyl-22,23-dihydro C-076 B1a and 5.8 mg of 4",5-di-O-acetyl-22,23-dihyro C-076 B1a after lyophilization from benzene. The structures are confirmed by 300 MHz nuclear magnetic resonance and mass spectrometry.

EXAMPLE 11

22,23-Dihydro C-076 B1a

39 G. of C-076 B1a is dissolved in 1540 ml. of toluene and introduced into a 4 liter stirred autoclave. To this is added 3.9 g. of tris (triphenylphosphine) rhodium (I) chloride (Wilkinson's catalyst). A hydrogenation pressure of 40 psi. and a temperature of 40° C. is maintained with stirring for 4½ hours. At the end of this period liquid chromatographic analysis indicates 98% yield of dihydro C-076 B1a with 1.5% of tetrahydro C-076 B1a. The toluene is removed by evaporation in vacuo and the dark red gum is dissolved in ethanol at a rate of 4 ml. of ethanol per gram of product. Formamide at a rate of 10 ml. per gram of product is added and the solution heated on the steam bath to 40°–50° while added water at a rate of 2 ml. per gram of product. After crystallization commences the heat is removed and the solution allowed to cool slowly with stirring overnight. The solid is filtered off and washed with a mixture 3 parts water and 1 part ethanol and dried in vacuo overnight. The solids are dissolved in 150 ml. of ethanol and warmed to 35°–40° C. on the steam bath. Water, 150 ml. is added slowly with stirring. When solution is complete at 35° C. the heat is removed and the solution allowed to cool slowly overnight. The crystals are removed by filtration and washed with 50% aqueous ethanol and dried in vacuo overnight affording 32.55 g. of 22,23-dihydro C-076 B1a with a m.p. of 155°–157° C.

PREPARATION 1

A 250 ml. baffled Erlenmeyer flask containing 50 ml. of the following medium:

Lactose	2.0%
Distiller's soluble	1.5%
Autolyzed yeast, Ardamine pH	0.5%
pH - before sterilization	7.0

is inoculated with the contents of one frozen vial of *Streptomyces avermitilis* MA 4848 and incubated on a rotary shaker at 28° C. for 24 hours at 150 RPM.

10 Ml. of the above fermentation media is employed to inoculate 500 ml. of the same medium as above in a 2 liter baffled Erlenmeyer flask. The fermentation media is incubated at 150 RPM on a rotary shaker at 28° C. for 24 hours.

All of the foregoing media is employed to inoculate 467 liters of the following media in a 756 liter stainless steel fermentor:

Lactose	2.0%
Distiller's solubles	1.5%
Autolyzed yeast, Ardamine pH	0.5%
Polyglycol 2000	0.32 ml./liter

-continued

pH - before sterilization	7.0

The fermentation media is incubated at 28° C. for 40 hours with an air flow 10 cubic feet per minute and an agitation rate 130 RPM.

230 Liters of the above media is employed to inoculate 4,310 liters of the following medium in a 5,670 liter stainless steel fermentor:

Dextrose	4.5%
Peptonized milk	2.4%
Autolyzed yeast, Ardamine pH	0.25%
Polyglycol 2000	2.5 ml./liter
pH - before sterilization	7.0

The fermentation continues for 144 hours at 26° C. with an air flow rate of 54.3 cubic feet per minute and agitation of 120 RPM.

The fermentation media are filtered and the mycelial filter cake washed with about 550 liters of water, the filtrate and washings are discarded. The filter cake is agitated with about 1500 liters of acetone for about one hour and filtered. The filter cake is washed with a mixture of about 150 liters of acetone and 40 liters of deionized water affording about 2000 liters of extract.

The foregoing fermentation and extraction is repeated on the same scale affording a further 2000 liters of acetone extract which is combined with the first extract and evaporated to a volume of about 800 liters. The pH of the concentrate is adjusted to about 4.7 with concentrated hydrochloric acid and combined with about 800 liters of methylene chloride. The combined solvents are agitated for about 4 hours and separated. The aqueous layer is combined with an additional 800 liters of methylene chloride and agitated for about 4 hours. The layers are separated and each methylene chloride extract separately treated with about 10 kilograms of Super-Cel and filtered. Both extracts are evaporated to a combined volume of about 60 liters.

PREPARATION 2

The 60 liter solution of C-076 in methylene chloride of the previous example is concentrated to dryness in vacuo and the residue is combined 3 times with 60 liter portions of methanol and evaporated to dryness to remove any residual methylene chloride. The final methanol concentrate volume is approximately 36 liters. The methanol solution is stored overnight and filtered. The filter cake is washed with 40 liters of fresh methanol and the methanol filtrates and washings are combined. The methanol solution is combined with 95 liters of ethylene glycol and 130 liters of heptane. The 2 layer solution is agitated for 5 minutes and the lower layer (ethylene glycol and methanol) is separated. The heptane solution is washed with a mixture of 20 liters of ethylene glycol and 6.3 liters methanol. After five minutes of agitation, the lower layer is separated and combined with the first ethylene glycol/methanol extract. An equal volume of water (approximately 150 liters) containing 79 g. of salt per liter is added to the ethylene glycol/methanol extracts. This solution is extracted with 150 liters of ethyl ether with agitation for 5 minutes. The ether layer is washed with 75 liters of water (½ volume) and agitated for 5 minutes and the layers separated. This procedure is repeated an additional 2 times (the final water wash contains 20 g. of salt per liter) affording a final ether layer volume of 110 liters. The ether layer is concentrated in vacuo, to a minimum volume, keeping the temperature less than 25° C. 40 Liters of methylene chloride is added to the residue and the solution is evaporated to dryness. This procedure is repeated and the final residue concentrated in vacuo at 50° C. to dryness.

PREPARATION 3

A 30 centimeter diameter column is prepared with a layer of 34 kilograms of activated alumina followed by a layer of 34 kilograms of activated carbon in a solution of methylene chloride. The residue from the previous example is dissolved in methylene chloride to a volume of 34 liters and applied to the column and eluted with 34 liters of methylene chloride. these fractions are discarded. A 3% solution of isopropanol and methylene chloride (20.8 liters of isopropanol and 660 liters of methylene chloride) is applied to the column and eluted in approximately 200 liter fractions. The combined isopropanol and methylene chloride fractions are evaporated in vacuo at a bath temperature of about 60° C. to a volume of about 20 liters. The bath temperature is reduced to about 45° C. and the extract is evaporated to dryness in vacuo. The residue is dissolved in 10 parts methylene chloride, 10 parts hexane and one part methanol to a final volume of 15 liters. This solution is applied directly to the Sephadex LH-20 column of the next example.

PREPARATION 4

A 30 centimeter diameter column is prepared in methanol with 36 kilograms of Sephadex LH-20 (available from Pharmacia Fine Chemicals, 800 Centennial Avenue, Piscataway, N.J. 08854) and washed with a solvent consisting of 10 parts methylene chloride, 10 parts hexane and one part methanol. One-fourth of the C-076 solution of Example 10 is applied to the column and the column eluted at a rate of 250 ml. per minute. Two 20 liter forecuts are collected and discarded followed by 20 two liter rich cuts (identified as fractions 1–20), followed by a single 20 liter tail cut, which is discarded. Fractions 1–8 are found to contain the C-076 A compounds and fractions 9–20 are found to contain the C-076 B compounds.

PREPARATION 5

The process of Preparation 4 is repeated on the same scale three more times and all of the fractions containing the C-076 B components (fractions 9–20) are combined and evaporated to dryness, affording 818 g. of crude mixed C-076 B components. The sample is found to contain 55% C-076 B1 and 39% of C-076 B2. 680.5 G. of this sample is dissolved in 2 liters of methylene chloride and placed in a 22 liter three neck round bottom flask followed by the addition of 13.6 liters of methanol. The methylene chloride is removed by distillation. 13.6 Liters of ethylene glycol is added as the methanol is being distilled under reduced pressure. The rate of distillation is maintained such that the temperature of the solution did not go below 65° C. When the addition of the ethylene glycol is complete, the solution is allowed to cool at 5° C. for sixteen hours. The crystals are filtered and washed with 1 liter of cold ethylene glycol. The crystals are then redissolved in 2 liters of methylene chloride the solution placed in a 22 liter three necked round bottom flask. The procedure described above is repeated twice. The first time 12.5 liters each of

methanol and ethylene glycol is employed and the second time 13.6 liters each of methanol and ethylene glycol is employed. The final crystals are washed with 1 liter of cold ethylene glycol and 1 liter of water. The crystals are dissolved in 4 liters of water and dried by filtering through sodium sulfate. The benzene solution is concentrated to a volume of 2 liters and lyophilized affording 241.2 gm. of a white powder consisting of 98% C-076 B_1 and 1% of C-076 B_2.

The mother liquors (22 liters) from the first two crystallizations above are combined and diluted with 22 liters of water. The aqueous solution is extracted with 60 liters of toluene and again with 15 liters of toluene. The toluene extract is then washed with 48 liters of water. The organic phase is filtered through Super-Cel to remove any residual water and evaporated affording 336 gm. of solid material consisting of 79% C-076 B_2 and 16% C-076 B_1 compounds.

PREPARATION 6

In the four Sephadex LH-20 columns of the procedure of Preparation 4, fractions 1–8 contain the C-076 A compounds and are combined. By HPLC analysis the mixture is found to contain 252 g. of C-076 A2a, 16 g. of A2b, 94 g. of A1a and 24 g. of A1b. The material is dissolved in a solvent system consisting of hexane:-toluene:methanol in the proportion of 6:1:1 and applied to the Sephadex LH-20 column of the same dimensions as the one used in Preparation 4 in the above solvent. Fractions are collected at the rate of 250 mL per minute and a 20 liter forecut is collected and discarded. Further elution affords 2 additional 20 liter forecuts which are also discarded and 50 four liter rich cuts which contain C-076 A compounds. Fractions 3–8 are found to contain predominately C-076 A1 components (40.2 g. A1a and 6.7 g. A1b), and fractions 29–36 are found to contain C-076 A2 compounds (117.2 g. A2a and 7.35 g. of A2b). Fractions 9–28 contain a mixture of C-076 A1 and A2 compounds.

PREPARATION 7

A sample of 150 g. of C-076 B1 from Preparation 5 is dissolved in 3 liters of a solvent mixture of hexane:-toluene:methanol in the ratio of 3:1:1. The solution is passed through a column of Sephadex LH-20 (of the same dimensions as the one used in Preparation 4) in the above solvent taking fractions at the rate of 250 ml. per minutes. After two 20 liter portions of the solvent mixture are collected and discarded, forecut of 10 liters is taken and discarded. Then 30 richcuts of 2 liters each are taken. Fractions 1–13 and 25–30 are discarded. Fractions 14–16 are combined and contain 80 g. of predominately C-076 B1a. Fractions 22–24 are combined and contain 6.7 g. of predominately C-076 B1b. Fractions 17–21 contain a mixture of C-076 B1a and B1b.

Fractions 17–21 above are combined and concentrated and passed through a Sephadex LH-20 column with the same solvent system as above. Three 20 liter forecuts are taken and discarded. Richcuts are then taken as follows: 5 cuts of 2 liters each (fractions 1–5); 20 cuts of 1 liter each (fractions 6–25); and 10 cuts of 2 liters each (fractions 26–35). Fractions 1–15 are discarded; fractions 16–21 contain 13.5 g. of C-076 B1a and 0.4 g. of C-076 B1b; fractions 22–26 contain 44 g. of C-076 B1a and 0.13 g. of C-076 B1b; fractions 27–30 contain 10.2 g. of C-076 B1a and 0.8 g. of C-076 B1b.

PREPARATION 8

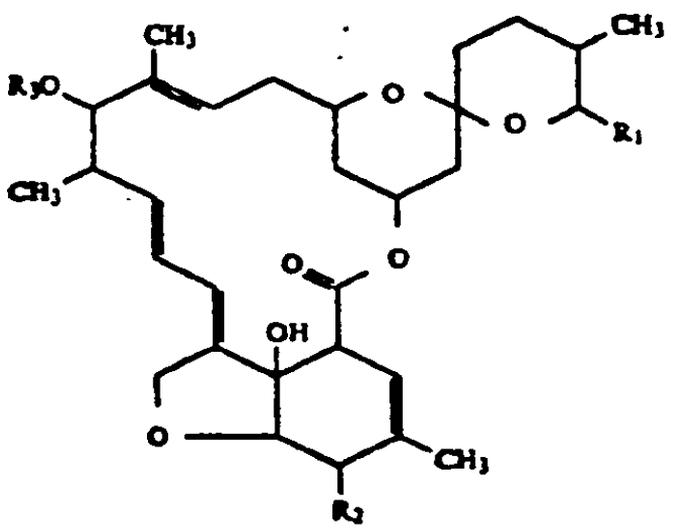
A mixture of all 8 C-076 components are chromatographed on a high pressure liquid chromatography column 4 mm. × 30 cm. packed with 10 micron μ Bondapak C_{18} silica gel (available from Waters Associates inc., Maple Street, Milford, Massachusetts 01757) eluting with 85:15 (v/v) methanol:water at a constant 40° C. At a flow rate of 1.2 mL per minute all eight compounds are separated and the elution volumes, which under the foregoing constant conditions are characteristic of the individual compounds are as follows:

	Elution Volume (Ve) Ml
C-076 B_2b	5.90
C-076 B_2a	6.52
C-076 A_2b	7.12
C-076 A_2a	7.88
C-076 B_1b	8.36
C-076 B_1a	9.60
C-076 A_1b	10.24
C-076 A_1a	11.88

The separation of C-076 "b" components from the respective "a" components is accomplished using techniques such as high pressure liquid chromatography. An absolute methanol solution of 30 microliters of a mixture of C-076 A1a and A1b, estimated to contain 30 micrograms of C-076 A1b is placed on a 3×250 mm. high pressure liquid chromatography column containing Spherisorb 5 micron ODS (available from Spectra Physics) as packing. The column is eluted with 85:15 methanol-water at a rate of 0.15 mL/min. The elution of the products are followed by observing the ultraviolet absorption of the eluent and collecting the individual components at the outlet of the UV monitor. 30 Micrograms of C-076 A1b is recovered in this manner.

What is claimed is:

1. A compound having the formula:



wherein

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy, hydroxy or loweralkanoyloxy; and

R_3 is hydrogen; loweralkanoyl; α-L-oleandrosyl; 4'-loweralkanoyl-α-L-oleandrosyl; 4'-(α-L-oleandrosyl)-α-L-oleandrosyl, 4''-loweralkanoyl-4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

2. The compound of claim 1 wherein:

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy or hydroxy; and

R_3 is hydrogen, α-L-oleandrosyl or 4'-(α-L-oleandrosyl)-α-L-oleandrosyl.

3. The compound of claim 2 wherein R_1 is iso-propyl.

4. The compound of claim 2 wherein R_1 is sec-butyl.

5. The compound of claim 4 which is 22,23-dihydro-C-076 A1a.

6. The compound of claim 4 which is 22,23-dihydro-C-076 B1a.

7. The compound of claim 4 which is 22,23-dihydro-C-076 A1a aglycone.

8. The compound of claim 4 which is 22,23-dihydro-C-076 B1a aglycone.

9. The compound of claim 4 which is 22,23-dihydro C-076 A1a monosaccharide.

10. The compound of claim 4 which is 22,23-dihydro C-076 B1a monosaccharide.

11. The compound of claim 1 wherein the lower-alkanoyl group of R_3 is acetyl.

12. The compound of claim 11 which is 4"-O-acetyl-22,23-dihydro C-076 A1a.

13. The compound of claim 11 which is 4"-O-acetyl-22,23-dihydro C-076 B1a.

14. The compound of claim 11 which is 4", 5-di-O-acetyl 22,23-dihydro C-076 B1a.

15. A method for treating for parasites which comprises treating the animal or area infected with parasites with an effective amount of one or more compounds of claim 1.

16. The method of claim 15 wherein the active compound is C-076 B1a.

17. The method of claim 15 wherein the active compound is a mixture of about 80% C-076 B1a and 20% C-076 B1b.

18. A composition for the treatment of parasitic infections which comprises an inert carrier and one or more compounds of claim 1.

19. The composition of claim 18 wherein the active compound is C-076 B1a.

20. The composition of claim 18 wherein the active compound is a mixture of about 80% C-076 B1a and 20% C-076 B1b.

* * * * *

ATTACHMENT

B

STROMECTOL® (Ivermectin)
Chronology of Events

Date	Event
12/1/89	Start of Gentilini Clinical Study, Protocol 004 (a non-IND study).
7/16/90	First Case Report Form received in Gentilini Clinical Study.
7/17/90	Original IND (35,136) is submitted to FDA.
7/26/90	Chemistry and Manufacturing Amendment is submitted to Agency regarding composition, method of manufacture and control, and sample label copy for Ivermectin Tablets, 6mg.
8/30/90	Start of Gann Clinical Study, Protocol 015.
11/5/90	Start of Burk Clinical Study, Protocol 014.
5/1/91	Start of Dreyer Clinical Study, Protocol 020 (a non-ID study).
7/30/91	Last Case Report Form received in Gentilini Clinical Study.
8/19/91	First Case Report Form received in Burk Clinical Study.
9/17/91	First Annual IND Progress Report is submitted to FDA.
4/7/92	First Case Report Form received in Dreyer Clinical Study.
5/28/92	Last Case Report Form received in Dreyer Clinical Study.
6/5/92	First Case Report Form received in Gann Clinical Study.
9/3/92	Second Annual IND Progress Report is submitted to FDA.
10/29/92	Last Case Report Form received in Gann Clinical Study
12/11/92	Background Package for Pre-NDA meeting is submitted to FDA.
12/29/92	Last Case Report Form received in Burk Clinical Study.

STROMECTOL® (Ivermectin)
Chronology of Events

Date	Event
1/6/93	MRL had a pre-NDA meeting with the FDA. At that time, FDA encourages us to include data from all treatments for strongyloides (such as compassionate treatments, overseas data, publications, etc.). At the same time it was decided that the claim could not be filed under Orphan Drug Regulations because of the need to include Puerto Rico, Guam, and other US territories.
2/11/93	MRL minutes to the 1/6/93 pre-NDA meeting is submitted to FDA. CDER did propose alternatives to enhance the existing data and encouraged MRL to file the NDA with consideration to their recommendations. The claim will not be filed under Orphan Drug Regulations.
9/17/93	Third Annual IND Progress Report is submitted to FDA.
12/13/93	Letter is submitted to Agency to inform them that MRL is suspending plans to file an NDA for the treatment of strongyloidiasis. MRL asks the Agency if they would consider adopting an EC concertation philosophy since the French reviewed and approved this claim and review the French dossier.
12/28/93	MRL requests authorization to export ivermectin bulk drug to Holland for formulation into tablets, which are shipped to France for final packaging for donation for human use for the treatment of Onchocerciasis.
6/16/94	MRL response to request for information regarding export report. Attached are two letters of approval for MECTIZAN® dated 10/15/87 and copies of the French approval letters translated to English.
9/29/94	Fourth Annual IND Progress Report is submitted to FDA.
5/3/95	Pre-NDA background package is submitted to FDA. Inform CDER of the interest of MRL to file an NDA for the use of Ivermectin in the treatment of both Strongyloides and Onchocerciasis.
7/20/95	Chemistry and Manufacturing Amendment is submitted to the Agency regarding 6mg French Blister Pacs.

STROMECTOL® (Ivermectin)
Chronology of Events

Date	Event
8/9/95	Preclinical Pharmacology and Toxicology information is submitted to the Agency. (Exploratory Acute Oral Toxicity Study in Mice TT #94-2800).
9/15/95	Draft Clinical Study Report prepared for Dreyer Clinical Study.
9/29/95	Draft Clinical Study Report prepared for Gentilini Clinical Study.
11/2/95	Letter is sent from Merck to the Agency submitting the Chemistry, Manufacturing and Controls proposal as indicated during a phone conversation between MRL and FDA on 11/i/95.
11/5/95	Draft Clinical Study Reports prepared for Gann and Burk Clinical Studies.
11/6/95	Fifth Annual IND Progress Report submitted to FDA.
2/2/96	Clinical Study Report approved for Dreyer, Gann and Burk Clinical Studies.
2/7/96	Clinical Study Report approved for Gentilini Clinical Study.
3/29/96	New Drug Application (20-721) is submitted to FDA (NDA 20-721 changed on 7/23/96 to NDA 50-742).
4/16/96	Letter is sent from Merck to FDA containing a listing of 25 countries in which MECTIZAN® is approved and the Physicians Circulars that are currently being used in each. (MECTIZAN® is ivermectin for the treatment of onchocerciasis under the Merck donation program to countries endemic with river blindness)
5/22/96	Letter is sent from Merck to FDA that contained the addresses of the manufacturing sites of both bulk drug substance and finished tablets.

STROMECTOL® (Ivermectin)
Chronology of Events

Date	Event
5/23/96	"60 Day" Teleconference meeting is held with FDA. MRL is informed by FDA that the NDA for Ivermectin is fileable for both indications, strongyloidiasis and onchocerciasis.
6/28/96	Letter is sent from Merck to FDA regarding Serious Adverse Experiences at a nursing home in Canada. (These studies were carried out under a non-IND compassionate use protocol.
7/9/96	As requested by the agency at the "60 day" teleconference, Patient Line Listings from the original and supplemental MAA were sent to the FDA.
7/16/96	As requested by FDA Biopharmaceutics Reviewer, responses to 5 questions were sent to FDA.
7/19/96	Summary reports by the French Agency on the original and supplemental Marketing Authorization Applications (MAA's) are sent to FDA as requested at the "60-Day" teleconference meeting on May 28, 1996.
7/22/96	Letter sent to FDA to address Dr. Colangelo's (FDA) concerns regarding determination of the half-life of ivermectin as requested at a teleconference held on 6/14/96 between MRL and FDA.
7/23/96	MRL received a Fax containing comments from the FDA chemist.
7/23/96	The NDA number for ivermectin has been changed from "20-721" to "50-742" per a telephone conversation between Mr. Frank Ricci (MRL) and Ms. Pauline Fogarty (FDA). This change was required because of the change in classification from 505 to 507.
7/24/96	Letter is sent to Mr. Falcone (FDA) regarding a listing of Ivermectin Tablet, bulk drug substance, analytical columns and other supportive documentation requested for submission to FDA for Methods Validation testing.
7/31/96	Four month Safety Update Report (Item 9) is sent to FDA.

STROMECTOL® (Ivermectin)
Chronology of Events

Date	Event
8/1/96	Telephone conversation between Dr. Brown (MRL) and Dr. Mathew Thomas (FDA, Clinical Investigations Branch, Pharmacologist) regarding finalization of plans to inspect the Gentilini/Datry site from September 16-20, 1996.
8/6/96	Diskette containing the 3 clinical study reports is sent to FDA for their use in reviewing the Clinical Documentation portion of the NDA and preparing the clinical assessment report for ivermectin.
8/14/96	Case report forms sent to Dr. Mathew Thomas (FDA, Clinical Investigations Branch) for use in the inspections taking place at the Gentilini study in Paris.
8/16/96	Teleconference is held between MRL and FDA regarding the statistical Review of NDA 50-742. In addition, at the same time Merck was advised that NDA 50-742 is on a priority review.
8/23/96	As requested at the 8/16/96 teleconference, ASCII files for the strongyloidiasis clinical studies (Protocols 004 (Gentilini),014 (Burk),015 (Gann), and 020 (Dreyer) are sent to FDA.
8/27/96	FDA request of materials for inspection of Dr. Gentilini's site in Paris are sent to Ms. B.J. Marciante of the FDA who will be working with Dr. Mathew Thomas (FDA, Clinical Investigations Branch) in the inspection.
8/28/96	Responses to questions from Dr. Coyne (sent to MRL via fax on 8/16/96) are submitted to FDA. These responses were provided by Dr. Marti of WHO.
8/28/96	Responses to 13 Chemistry Issues and 4 Microbiology issues which were requested via fax on 7/23/96 from Ms. Pauline Fogarty (CSO) are sent to FDA.